Chemical Name: Afidopyropen
USEPA PC Code: 026200
USEPA MRID: 49689224
USEPA DP Barcode: 435146
PMRA Data Code (DACO): 9.2.4.8
PMRA Study No. (UKID): 2627488
Data Requirement: OCSPP Guideline 850.3030

Test Material: BAS 440 00 I (TEP, VERSYS™) **Purity:** 9.7%

Active Ingredient: Afidopyropen
IUPAC Name: [(3*S*,4*R*,4a*R*,6*S*,6a*S*,12*R*,12a*S*,12b*S*)-3-(cyclopropylcarbonyloxy)-1,2,3,4,4a,5,6,6a,12a,12b-decahydro-6,12-dihydroxy-4,6a,12b-trimethyl-11-oxo-9-(3-pyridyl)-11*H*,12*H*-benzo[*f*]pyrano[4,3-*b*]chromen-4-yl]methylcyclopropane carboxylate
CAS Name: [(3*S*,4*R*,4a*R*,6*S*,6a*S*,12*R*,12a*S*,12b*S*)-3-(cyclopropylcarbonyl)oxy)]-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-6,12-dihydroxy-4,6a,12b-trimethyl-11-oxo-9-(3-pyridyl)-2*H*,11*H*-naphtho[2,1-*b*]pyrano[3,4-*e*]pyran-4-yl]methyl cyclopropanecarboxylate
CAS No.: 915972-17-7
Synonyms: INSCALIS™

Primary Reviewer: Moncie V. Wright
Environmental Scientist, CDM Smith/CSS-Dynamac JV
Signature:
Date: 9 November 2016

Secondary Reviewer: Teri Myers, Ph.D.
Senior Scientist, CDM Smith/CSS-Dynamac JV
Signature:
Date: 28 November 2016

EFED Reviewer: Cameron Douglass, Ph.D.
Biologist, USEPA/OCSPP/OPP/EFED/ERBIV
Signature: Cameron Douglass 2018.02.13 13:01:26 -05'00'
Date: 13 February 2018

PMRA Reviewer: Vedad Izadi
Date: 11 September 2017

Date Evaluation Completed: 11 September 2017

CITATION:
T Leak. 2015. BAS 440 00 I (a.i. name/reg. no. Afidopyropen/5599022): Toxicity of residues on foliage to the honeybee, *Apis mellifera*. Study conducted by ABC Laboratories, Columbia, Missouri. Laboratory study code: 81148. Study sponsored by BASF Corporation, Research Triangle Park, North Carolina. BASF study code: 721401. Study initiated 4 June 2014 and completed 27 January 2015.

Executive Summary:
Adult worker honeybees (*Apis mellifera* L.) were exposed to the formulated end-use product BAS 440 00 I (VERSYS™, 9.7% afidopyropen) for 24 hours in a foliar residue test. Nominal application rates to alfalfa were 0 (negative control) and 0.045 lb a.i./A (50.0 g a.i./ha); alfalfa plants were then placed outdoors to weather. Measured rates were 0.047, 0.044, 0.043, and 0.047 lb a.i./A (52.6, 49.8, 48.2, and 52.6 g a.i./ha) after 0, 3, 8, and 24 hours weathering, respectively; measured rates represent 104.3, 98.8, 95.6,

and 104.3%, respectively, of the nominal application rate of 0.045 lb a.i./A. Measured levels in the negative control never exceeded the minimum quantifiable limit (MQL, <0.00001).

Resulting afidopyropen foliar residue levels on alfalfa weathered for 0, 3, 8, and 24 hours, respectively, were 2.89, 1.42, 1.15, and 1.56 mg a.i./kg. Honeybee mortality 4 hours after exposure to the treated foliage was 0.7, 0.7, and 1.3%, respectively, for foliage weather for 3, 8, and 24 hours. Honeybee mortality 24 hours after exposure to the treated foliage was 6.0, 6.7, and 8.7%, respectively, for foliage weather for 3, 8, and 24 hours. While in most treatment groups no sublethal behavioral effects were reported, in the test item treatment group that was allowed to weather for 24 hours, 2.0% of surviving bees were reported to be lying on their backs, and 1.3% of surviving bees displayed symptoms of lethargy.

The time required for weathered residues to be toxic to <25% of the bees (*i.e.*, the RT_{25} value) after 24 hours of weathering was < 3h for adult honeybees under the conditions tested.

Results Synopsis:

24-h $RT_{25:}$ < 3h

EPA Classification: Acceptable
PMRA Classification: Fully reliable

I. DATA SOURCE

USEPA MRID No.:	49689224
PMRA Study No.:	2627488
Study Title:	BAS 440 00 I (a.i. name/reg. no. Afidopyropen/5599022): Toxicity of residues on foliage to the honeybee, *Apis mellifera*.
Study Author(s):	T Leak
Testing Laboratory:	ABC Laboratories, Columbia, Missouri
Laboratory Report No.:	81148
Sponsor Study No.:	721401
Study Completion Date:	January 27, 2015
Data Access:	Data submitter is data owner
Data Protection Claimed:	Yes; no claim of confidentiality was made.

II. MATERIALS AND METHODS

Test Guideline: OCSPP Guideline 850.3030

Deviations from Guideline:

No guideline deviations were noted by the study author; the following deviations and other study deficiencies were noted by the reviewers:

- The OCSPP 850.3030 guideline validity requirements states that environmental conditions at the field site where treated crops are located for weathering be reported, and these details were not provided for this particular study; moreover, it's not clear that environmental conditions at the field location were even monitored.

- No details are given in the study report on lighting conditions in the area of the laboratory where bee test cages are kept; the OCSPP 850.3030 guideline recommends that bees be maintained in the dark during the exposure period.

GLP Compliance: Yes; study conducted in compliance with the U.S. EPA Good Laboratory Practice Standards (40 CFR Part 792), with the exception that the latest (April 2014) characterizations of the laboratory's well water were not performed in accordance with the stated GLP standards.

A. MATERIALS

Test Material: BAS 440 00 I (TEP, VERSYS™), 9.7% (w/w)
Test Material Identity: Yellow liquid; batch no. 1767-104-1

Details on Preparation and Application of Test Materials:

The test included a negative control (deionized water) only. The test substance spray solution was prepared by transferring 0.322 g of BAS 440 00 I to a 250 mL volumetric flask, and bringing the flask to volume with DI water to reach the target concentration of 0.125 mg a.i/mL. An overhead track sprayer (De Vries Manufacturing) was equipped with a TeeJet 4004 E nozzle and operated at 40 psi. Flats of the test plants (alfalfa) were sprayed with either water alone, or the treated spray solution; negative control applications were made first. After applications, flats were transported to a controlled outdoor location to facilitate aging (weathering). Three treated flats and one control flat were sprayed for each weathering period. For T0 samples foliage was allowed to dry for 30 minutes before collection. The top 15 cm of alfalfa plants in the middle of the flats were cut with clippers, and the foliage was composited by aging period into plastic bags that were sealed for transport back to the laboratory. Samples were subsequently chopped, mixed, and subsamples (15 g) collected. The 15 g subsamples of alfalfa were placed in the center of each bee cage (1 per cage), following anaesthetization of the bees and centering of the bees in each cage. It's not entirely clear from the study author's description of the definitive test where the bees were located relative to the foliage introduced into the test cages; the study author reported that the approximate elapsed time from foliage sampling to exposure of bees to the foliage was 1.5 hours.

Analytical Monitoring: Analytical verification of the applied test material was performed for the spray solutions and treated alfalfa foliage.

Details on Analytical Method: LC-MS/MS
Reference Material: TG dimethoate (99.2%)
Reference Material Identity Lot no. 189700
Vehicle: N/A

Test Organism (Species): *Apis mellifera* L. (honeybee)
Animal Group: Arthropoda/Insecta/Hymenoptera/Apidae

Details on Test Organisms: Adult worker bees of roughly similar ages - bees were shaken from the frame, so were all adults - were obtained from a commercial apiary (C. Gibbons Honey, Columbia, Missouri). The bees used in the test were from a single, disease-free colony that had never been treated for *Varroa* mites or for disease. The bees were extracted from the frames by shaking the frames over a transport box, and were added to the cages on the day of test initiation. The bees were maintained in a clean holding cage within an environmental chamber (25°C and 50-70% relative humidity). Prior to test initiation, the bees were cleaned with solvent, then impartially selected and transferred to the test cages after anaesthetization with CO_2.

B. STUDY DESIGN AND METHODS

Study Type: Laboratory
Test Duration Type: Acute
Limit Test: N/A
Total Exposure Duration: 24 hours
Post-Exposure Observation Period:
N/A
Remarks: Following extraction, residue samples were analyzed via liquid chromatography/tandem mass spectroscopy (LC-MS/MS; AbSciex API-550); specifically using a Waters Acuity® Ultra-high Performance Liquid Chromatography (UPLC) Ethylene Bridged Hybrid (BEH) C18 column held at 50°C. Mobile phase A was 0.1% formic acid, and mobile phase B was 0.1% formic acid in acetonitrile; the mobile phase gradient was 80:20 (%A/%B) for 1.6 min, 50:50 for 1.4 min, 1:99 for 1.5 min, and 80:20 for 1.5 min. Quality control (QC) samples (to test recovery of the test item from treated foliage) were prepared by spiking foliage collected from negative control-treated plants with 0.13 and 130 mg a.i./kg of the test item. Test item concentrations in spray solutions were also verified via collection of an approximately 10 µL sub-sample from each spray bottle immediately before applications were made, these QC samples were than analyzed on the LC-MS/MS.

Test Environmental Conditions: Temperature was 26.3 to 26.6°C, and relative humidity 43 to 52% in the area in which test cages were kept.
Photoperiod and Lighting: Not reported.
Feeding: Bees were provided 50% (w/v) sucrose solution *ad libitum* via a sucrose solution-saturated dental cotton roll. Bees were fed during holding, acclimation, and testing.

Nominal and Measured Concentration:
Nominal application rate: 0 (negative [water] control) and 0.045 lb a.i./A (50 g a.i./ha)
Measured application rate: 0.047, 0.044, 0.043, and 0.047 lb a.i./A (52.6, 49.8, 48.2, and 52.6 g a.i./ha) at 0, 3, 8, and 24 hours,

	respectively; residues in the negative control never exceeded the MQL (<0.00001 lb a.i./A [<0.016 g a.i./ha])
Test Units:	Plastic, screened bee cages (14 x 19 x 10; width x length x height), cleaned with a solvent before use.
Test Design:	Worker bees were exposed to a negative control or one afidopyropen application rate via foliar residues that were permitted to weather for 0, 3, 8, and 24 hours after foliar application of the test material; a standardized reference toxicant test was also performed with dimethoate. Six replicate cages were used for each treatment (*i.e.*, negative control, test item at 0, 3, 8, and 24 hours weathering), with 25 bees in each replicate cage (total of 150 bees per control and treatment). Observations of mortality and sublethal behavioral abnormalities were made at 0 (control), 4, and 24 hours.

III. APPLICANT'S REPORTED RESULTS AND DISCUSSION

Exposure Duration:	24 hours
Endpoint(s):	Mortality, sublethal behavioral effects
Effect Concentration:	24-h RT_{25}: <3 hours
Basis for Concentration:	Measured foliar residue levels
Effect Concentration Type:	Test material
Basis for Effect:	Mortality

Details on Applicant-Provided Results:

Exposure: Analytical method validation samples yielded mean recoveries of 104 and 108% for the nominal test item concentrations 0.13 and 130.00 mg a.i./kg, indicating that the analytical method analysis of foliar afidopyropen residues was adequate. Analysis of sprayer application volumes indicated that for applications made on 20 August 2014 (*i.e.*, 0- and 24-hour applications), the applied spray solution was 102.8% of the nominal calibrated application volume (400 L/ha); for applications made on 21 August 2014 (*i.e.*, 3- and 8-hour applications), the applied spray solution was 100.5% of the nominal calibrated application volume (400 L/ha).

Analysis of the spray bottle solutions collected immediately prior to applications yielded mean recoveries of 104, 99, 96, and 104%, respectively, of the nominal test item application rate (0.045 lb a.i./A [50 g a.i./ha]) for the solutions used to treat the 0, 3, 8, and 24-hour weathered alfalfa plants. Recoveries of the spiked quality control samples were 99-109% of the nominal concentrations (0.13 and 130 mg a.i./kg).

Analysis of the treated foliage samples yielded test item concentrations of 2.89, 1.42, 1.15, and 1.56 mg a.i./kg, respectively, at 0, 3, 8, and 24 hours post-spray; no test item residues exceeding the MQL (0.078 mg a.i./kg) were detected on any of the negative control-treated foliage.

Sublethal Behavioral Effects: All bees in the negative control treatment group appeared normal (see **Table 1**). In the test item treatment group that was allowed to weather for 3 hours, all bees appeared normal 4 hours after exposure, and 24 hours after exposure 2.0% of surviving bees were reported to be lying on their backs. In the test item treatment group that was allowed to weather for 8 hours, all bees appeared normal 4 and 24 hours after exposure. In the test item treatment group that was allowed to

weather for 24 hours, all bees appeared normal 4 hours after exposure, and 24 hours after exposure 1.3% of surviving bees in one of the treatment replicates displayed symptoms of lethargy.

In the dimethoate treatment, 0.0, 0.0, 3.3, 0.0, and 3.3%, respectively, of bees in the 0.000 (negative control), 0.000 (vehicle control), 0.020, 0.100, and 0.200 µg dimethoate/bee treatment groups reportedly displayed erratic behavior.

Mortality: In the negative control treatment group, there was 1.3% mortality 4 hours after exposure, and 2.2% mortality 24 hours after exposure (see **Table 1**). In the afidopyropen treatment group that was allowed to weather for 3 hours, there was 0.7% mortality 4 hours after exposure, and 6.0% mortality 24 hours after exposure. In the afidopyropen test group that was allowed to weather for 8 hours, there was 0.7% mortality 4 hours after exposure, and 6.7% mortality 24 hours after exposure. In the afidopyropen treatment group that was allowed to weather for 24 hours, there was 1.3% mortality 4 hours after exposure, and 8.7% mortality 24 hours after exposure.

In the dimethoate test, average mortality was 0.0, 0.0, 3.3, 90.0, and 96.7%, respectively, in the 0.000 (negative control), 0.000 (vehicle control), 0.020, 0.100, and 0.200 µg dimethoate/bee treatment groups.

Table 1. Average honeybee (*Apis mellifera*) mortality (mean ± SE) in the residue toxicity test with negative control, the reference toxicant dimethoate (99.2%), and the test item, BAS 440 00 I (9.7% afidopyropen), applied at 0.045 lb a.i./A (50 g a.i./ha) to alfalfa foliage that was allowed to weather for 3, 8, and 24 hours, and then provided to bees.

Weathering Period (hours)	**Bees** (n)	**Mortality** (%)		**Sublethal Effect Observations n (effect code)**[1]	
		4 h	**24 h**	**4 h**	**24 h**
Negative control	150	1.3 ± 0.8	2.2 ± 4.7	0	0
Test substance (afidopyropen)					
3	150	0.7 ± 0.7	6.0 ± 2.3	0	3ob
8	150	0.7 ± 0.7	6.7 ± 2.5	0	0
24	150	1.3 ± 0.8	8.7 ± 2.6	0	2l
Treatment (µg a.i./bee [nominal])	Reference toxicant (dimethoate)				
Negative control	30	N/A	0.0 ± 0.0	N/A	0
Vehicle control	30		0.0 ± 0.0		0
0.020	30		3.3 ± 3.3		1e
0.100	30		90.0 ± 5.8		0
0.200	30		96.7 ± 3.3		1e

[1] Sublethal behavioral effects were categorized by the study author as: 'ob' – living bee(s) on their back; or, 'l' – lethargy; 'e' – erratic behavior.

Applicant-Reported Statistics and Error Estimates

Statistical analyses were not performed by the study author on data from the foliar toxicity of residues test since mortality was less than 10% after 24 hrs. Data from the dimethoate test was analyzed in SAS® (SAS Institute, Cary, NC), with a median lethal dose (LD_{50}) estimated with the trimmed Spearman-Karber method.

The estimated 24-h LD_{50} for the reference toxicant was 0.049 (0.042-0.056) µg dimethoate/bee.

IV. OVERALL REMARKS, ATTACHMENTS

The applicant submitted a full study report (PDF document), an OECD-formatted summary document, and an Excel spreadsheet containing raw honeybee mortality data.

V. PRIMARY REVIEWER'S ANALYSIS AND CONCLUSIONS

Due to <10% honeybee mortality no additional statistical analyses were performed by the reviewer. Treatment means were confirmed by the reviewer, and the description of sublethal behavioral effects reported by the study author - and detailed above - were verified.

Reviewer's Statistical Verification:

Because there was a lack of measurable mortality in the foliar residue toxicity test (*i.e.*, maximum treatment-related mortality was 9%), the EFED reviewer determined the time to reduce residue toxicity to 25% bee (RT_{25}) qualitatively based on the shortest weathering period, i.e., 3 hours.

24-h $RT_{25:}$ < 3h

Reviewer's Comments:

The in-life phase of the study was conducted in the period between 21 and 22 August 2014. All core guideline validity requirements (OCSPP 850.3030) appear to have been met by this study, with the exception of monitoring and reporting of environmental conditions at the field location where treated plants were located for weathering. The reviewer's overall results for the residue toxicity tests are consistent with those of the study author and are presented in the Executive Summary and Results Synopsis sections of this summary document.

This study with adult honeybees provides information on the potential toxicity of foliar residues resulting from an application of the formulated end-use product BAS 440 00 I (VERSYS™, 9.7% afidopyropen) of 0.045 lb a.i./A (50 g a.i./ha). Measured application rates on alfalfa weathered for 0, 3, 8, and 24 hours, respectively, were 0.047, 0.044, 0.043, and 0.047 lb a.i./A (52.6, 49.8, 48.2, and 52.6 g a.i./ha). Honeybee mortality 4 hours after exposure to treated foliage was 0.7, 0.7, and 1.3%, respectively, for foliage weather for 3, 8, and 24 hours. Honeybee mortality 24 hours after exposure to treated foliage was 6.0, 6.7, and 8.7%, respectively, for foliage weather for 3, 8, and 24 hours. Consequently, based on measured foliar residue levels, we can state that residue levels >0.044 lb a.i./A (>49.8 g a.i./ha) would likely be required to induce 25% mortality in honeybees exposed to those foliar residues.

The nominal afidopyropen application rate used in the study was 0.045 lb a.i./A (50 g a.i./ha), which is equivalent to the current proposed maximum single application rate.

The current OCSPP 850.3030 Guideline (rev. Jan. 2012) describes the treatment of crop foliage grown in the field, which is then harvested at the predetermined intervals (*i.e.*, 0, 3, 8, and 24 hours) post-application to allow for natural, field weathering of the treated foliage. In this study alfalfa was grown in a greenhouse in flats and then sprayed using a greenhouse spray chamber. The study report states that following applications the treated flats were located in a "controlled outdoor location," but no details

are given regarding the environmental conditions at this outdoor location and so it is not possible for the reviewer to evaluate how treated foliage might have weathered.

Reviewer's Conclusions:
Foliar residue levels after a 0.045 lb a.i./A (50 g a.i./ha) application of BAS 440 00 I (VERSYS™, 9.7% afidopyropen) to alfalfa, which was weathered for 0, 3, 8, and 24 hours, were 2.89, 1.42, 1.15, and 1.56 mg a.i./kg, respectively, respectively. Honeybee mortality 4 hours after exposure to the treated foliage was 0.7, 0.7, and 1.3%, respectively, for foliage weathered for 3, 8, and 24 hours. Honeybee mortality 24 hours after exposure to the treated foliage was 6.0, 6.7, and 8.7%, respectively, for foliage weathered for 3, 8, and 24 hours.

Results Synopsis

24-h $RT_{25:}$ <3h

EPA Classification: Acceptable
PMRA Classification: Fully reliable